Cell Host & Microbe
Article

Influenza Virus-Induced Glucocorticoids Compromise Innate Host Defense against a Secondary Bacterial Infection

Amanda M. Jamieson,[1,2] Shuang Yu,[1] Charles H. Annicelli,[1] and Ruslan Medzhitov[1,*]
[1]Howard Hughes Medical Institute and Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06510, USA
[2]Present address: Max F. Perutz Laboratories, Department of Microbiology, Immunobiology, and Genetics, University of Vienna, A-1030 Vienna, Austria
*Correspondence: ruslan.medzhitov@yale.edu
DOI 10.1016/j.chom.2010.01.010

SUMMARY

Multicellular organisms are continuously exposed to many different pathogens. Because different classes of pathogens require different types of immune responses, understanding how an ongoing immune response to one type of infection affects the host's ability to respond to another pathogen is essential for a complete understanding of host-pathogen interactions. Here, we used a mouse model of coinfection to gain insight into the effect of respiratory influenza virus infection on a subsequent systemic bacterial infection. We found that influenza infection triggered a generalized stress response leading to a sustained increase in serum glucocorticoid levels, resulting in a systemic suppression of immune responses. However, virus-induced glucocorticoid production was necessary to control the inflammatory response and prevent lethal immunopathology during coinfection. This study demonstrates that activation of the hypothalamic-pituitary-adrenal axis controls the balance between immune defense and immunopathology and is an important component of the host response to coinfection.

INTRODUCTION

Most studies of host-pathogen interactions have focused on the interaction of a single pathogen with a host cell or organism. However, host organisms in a natural environment are commonly exposed to multiple pathogens simultaneously. The immune response to infection can cause vast physiological alterations in the metazoan host, including changes in metabolism, serum proteins, cellular distribution, and hematopoiesis. In addition, the class of pathogen and the site of infection are instrumental in determining the extent and type of immune response induced. Therefore, an important problem in host-pathogen interactions is how the immune response to one pathogen alters the immune response to another infectious agent (Bakaletz, 2004). Polymicrobial infection, or coinfection, can lead to complications in the treatment of infectious diseases, and there are many well-recognized instances of coinfection that occur naturally in the human population (Beadling and Slifka, 2004; Elston and Thaker, 2008; Rénia and Potter, 2006). Several studies examining infection with bacteria during an ongoing response to a viral infection have found that, depending on the types of pathogens, the temporal proximity of the infections, and the route of infection, a variety of outcomes are possible (Barton et al., 2007; Gardner, 1981; Gumenscheimer et al., 2007; Humphreys et al., 2008; Navarini et al., 2006). Respiratory infection with influenza virus is particularly interesting in this regard, as it is most commonly complicated by secondary bacterial infections (Hament et al., 1999).

Influenza virus is a negative-stranded RNA virus that primarily infects the lung epithelium and leads to a strong local inflammatory response (La Gruta et al., 2007). Infection with influenza virus in humans usually leads to a brief but severe illness, and fatalities are often linked to coinfection with bacterial pathogens (Beadling and Slifka, 2004; Berendt and Jaax, 1985; Hament et al., 1999; McCullers, 2006; Peltola and McCullers, 2004; Seki et al., 2004; Sun and Metzger, 2008). Several studies have demonstrated that infection with influenza virus can result in immunosuppression and subsequent secondary bacterial infection (Borrow et al., 2005; Didierlaurent et al., 2008; Marcus et al., 2005; Noone et al., 2005; Shahangian et al., 2009; Sun and Metzger, 2008; Tumpey et al., 2000; van der Sluijs et al., 2004). However, these studies focused primarily on the local effects of influenza at the site of infection. The effect of influenza virus infection on the systemic immune response is less well understood.

Influenza virus infection facilitates the ability of bacterial pathogens to gain entry and replicate in the lung, and many of these pathogens are able to spread systemically with lethal complications (Beadling and Slifka, 2004). In order to specifically examine the effect of influenza virus lung infection on the systemic immune response to bacteria, we utilized the model bacterial pathogen *L. monocytogenes*. Although *L. monocytogenes* is not commonly associated with secondary bacterial infections during influenza infection, it was used here because it provides an excellent model of a systemic bacterial infection. The innate immune system is triggered rapidly after infection with *L. monocytogenes*, and it is crucial for early control of bacterial replication (Kaufmann, 1993). Two cytokines are important components of the innate immune response to *L. monocytogenes*, IL-6 and IFN-γ (Andersson et al., 1998; Dai et al., 1997; Dalrymple et al.,

1995; Kopf et al., 1994). The primary cells infected by *L. monocytogenes* are macrophages and hepatocytes (Selmi et al., 2007). Following infection, bacteria spread from cell to cell by co-opting the host cell's actin-based motility system (Portnoy et al., 2002). As with many systemic bacterial infections, the hepatic immune response is essential for the clearance of *L. monocytogenes* (Cousens and Wing, 2000; Selmi et al., 2007). The early immune response in the liver consists primarily of interactions between the resident hepatocytes and Kupffer cells and infiltrating monocytes and neutrophils (Gregory and Wing, 2002). While the innate immune response is able to control many aspects of *L. monocytogenes* infection, the adaptive immune system, and particularly the cytotoxic T cell response, is necessary for sterilizing immunity to the pathogen (Pamer, 2004; Unanue, 1997b).

We found that influenza infection suppressed the systemic antibacterial innate immune response. The major mechanism for this suppression was influenza virus infection-induced glucocorticoid (GC) production. GCs are produced by adrenal glands during times of psychological and physiological stress, and their production is regulated by the hypothalamic-pituitary-adrenal (HPA) axis (Di Comite et al., 2007; Rogatsky and Ivashkiv, 2006; Sternberg, 2006). They are known to have pleiotropic immunosuppressive effects and are important in regulating inflammation (Sternberg, 2006). Previous studies that demonstrated production of GCs during viral infection and other inflammatory immune responses proposed mechanisms of GC production caused by inflammatory cytokines such as IL-1 and IL-6 (Besedovsky and del Rey, 1989; Bethin et al., 2000; Dunn et al., 1989; Dunn and Vickers, 1994; Hermann et al., 1994; Ruzek et al., 1997). We show here that lung infection with influenza virus can induce GC production independently of elevated systemic inflammatory cytokines. Furthermore, we show that while GC production resulted in systemic immunosuppression, it was required to protect coinfected hosts from lethal immunopathology.

RESULTS

Coinfection with Influenza and *L. monocytogenes* Results in Increased Bacterial Burden

To examine the effect of an ongoing viral infection on the immune response to a systemic bacterial infection, C57BL/6 mice were infected intranasally with influenza virus and subsequently infected intravenously with *L. monocytogenes*. Bacterial and viral loads were measured in the liver and spleen 4 days after *L. monocytogenes* infection. Mice with a concurrent viral infection had a five-log greater bacterial load in the liver compared to mice infected with *L. monocytogenes* alone (Figures 1A and 1B). This increase in bacterial burden was dependent on the dose of influenza virus (Figure 1A). The minimal effective influenza virus dose caused an increase in bacterial cfu even when given as many as 5 days before *L. monocytogenes* (Figure 1B). A lower dose of virus (200 pfu) was effective in causing an increased bacterial load only when given 3–5 days before bacterial infection (Figure 1C and data not shown). Mice infected with a sublethal viral dose of 200 pfu 5 days before infection with *L. monocytogenes* also displayed delayed bacterial clearance when examined 8 days after bacterial infection (Figure 1C).

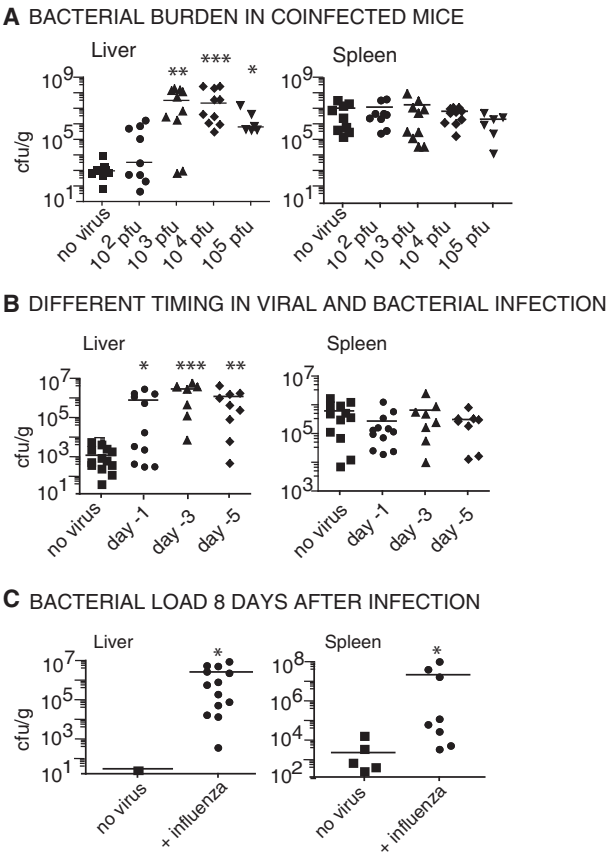

Figure 1. Bacterial Burden Is Increased in Influenza Virus-Infected Mice

(A) Mice infected with influenza virus were infected with *L. monocytogenes* 1 day later, and 4 days after bacterial infection, cfu per gram of organ were determined for the spleen and the liver.

(B) Mice were infected with 1000 pfu of influenza virus 1, 3, or 5 days before infection with *L. monocytogenes*, and cfu were determined 4 days after bacterial infection.

(C) Mice were infected with 200 pfu of influenza virus 5 days before infection with *L. monocytogenes*, and cfu were determined 8 days after bacterial infection. Data are from at least two independent experiments with at least four mice in each group (*$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$, no *$p > 0.05$). See also Figure S1.

Mice given this sublethal dose of influenza had elevated levels of bacteria in the liver 4, 6, 8, and 12 days after bacterial infection and in the spleen at the later time points (Figure S1). However, by 30 days after bacterial infection, the bacteria were cleared in both organs regardless of a previous infection with influenza virus (data not shown).

Type I IFNs have been shown to be detrimental to the host response during *L. monocytogenes* infection (Auerbuch et al., 2004; Carrero et al., 2004; Decker et al., 2005; O'Connell et al., 2004). Given that type I IFNs are produced during the early stages of an active influenza virus infection (Julkunen et al., 2001; Wang et al., 2000), it seemed possible that this was responsible for the increased cfu. However, type I IFNs were not responsible for the increase in bacterial cfu observed in coinfected mice, as there was still a significant increase in cfu in viral/bacterial coinfected *IFNαR1*-deficient mice (Figure 2A).

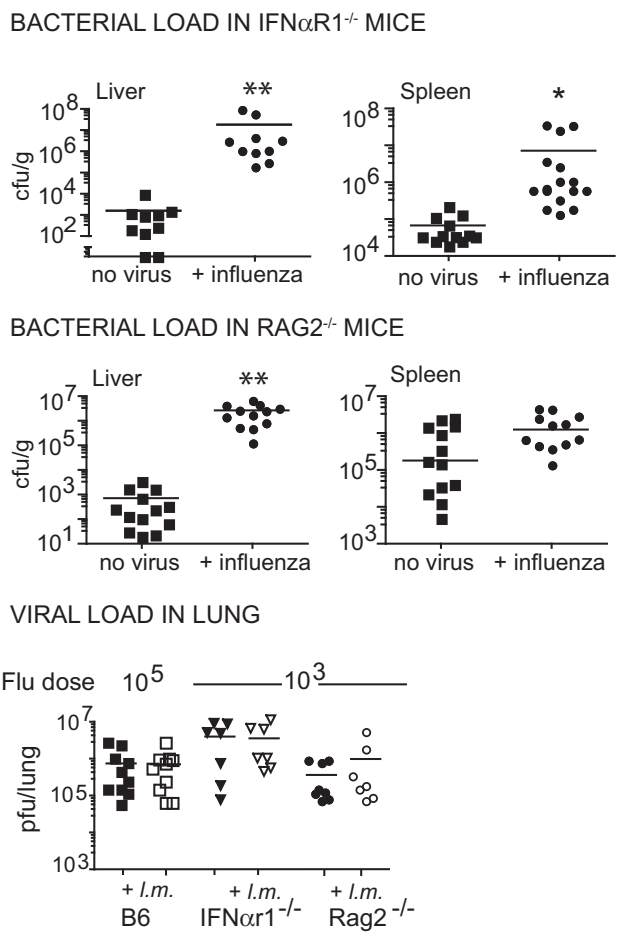

Figure 2. Bacterial Burden Is Increased in Coinfected *IFNαR1*$^{-/-}$ and *Rag2*$^{-/-}$ Mice

(A and B) Bacterial cfu were measured in the liver and spleen 4 days after infection with *L. monocytogenes* in *IFNαR1*$^{-/-}$ (A) or *Rag2*$^{-/-}$ (B) mice that were infected with 1000 pfu of influenza virus.

(C) Viral titers were measured in the lungs of C57BL/6 mice infected with 100,000 pfu and *IFNαR1*$^{-/-}$ or *Rag2*$^{-/-}$ mice that were infected with 1000 pfu of influenza virus. Data in (A) and (B) are from at least three independent experiments with at least three mice in each group, and data in (C) are from two independent experiments with at least three mice in each group (*$p \leq 0.05$, **$p \leq 0.001$, no *$p > 0.05$). See also Figure S2.

The increase in bacterial load caused by previous infection with influenza virus was observed as early as 4 days after infection with *L. monocytogenes*, suggesting a defect in the innate immune response. We examined further whether this increased bacterial burden was due to altered functions of the innate or acquired immune system by infecting *Rag2*$^{-/-}$ mice, which lack an acquired immune system. *Rag2*$^{-/-}$ coinfected mice exhibited increases in bacterial load that were similar to those observed in coinfected wild-type mice (Figure 2B), indicating that the acquired immune response was not responsible for the initial increase in cfu.

Since both type I IFNs and cells of the acquired immune system are necessary components of the antiviral immune response, both *IFNαR1*$^{-/-}$ and *Rag2*$^{-/-}$ mice had increased viral loads in the lungs when infected with the same dose of influenza as wild-type mice (data not shown). To compare the effect of influenza on subsequent bacterial infection in wild-type, *IFNαR1*$^{-/-}$, and *Rag2*$^{-/-}$ mice independent of this increased viral load, viral doses were used that resulted in similar viral loads in the lungs of all strains of mice (Figure 2C). As shown in previous studies (García-Sastre et al., 1998), influenza virus had altered tissue tropism in *IFNαR1*$^{-/-}$ mice and spread systemically to the kidneys (data not shown). While influenza virus infection had a profound effect on the bacterial burden, infection with *L. monocytogenes* did not alter the tissue tropism or viral titer of the influenza virus in comparison with singly infected controls (Figure 2C and data not shown).

Infection with Influenza Suppresses the Immune Response to *L. monocytogenes*

Since coinfection with influenza virus and *L. monocytogenes* led to increased bacterial burden, we next examined the immune response to *L. monocytogenes* after infection with influenza virus. One clear change was that the spleen size and total splenocyte number of both influenza virus-infected and coinfected mice was decreased compared to spleens of uninfected mice or mice infected with *L. monocytogenes* alone (Figure 3A). Systemic levels of inflammatory cytokines were also measured over the course of *L. monocytogenes* infection. When compared to mice infected with *L. monocytogenes* alone, the levels of IFN-γ and IL-6 in the serum were significantly decreased in coinfected mice (Figure 3B).

Since increased bacterial loads were observed mainly in the liver, we next directly examined the hepatic immune response. Recruitment of leukocytes to the liver by chemokines, cytokines, and adhesion molecules is essential for development of an appropriate immune response (Salmi et al., 1998). We therefore examined liver sections from mice 4 days postinfection with *L. monocytogenes* for recruitment of immune cells. There was a significant decrease in cellular infiltrate in the livers of coinfected mice compared to mice infected with *Listeria monocytogenes* alone (Figures 3C and S3A). At day 1 after bacterial infection, there was a significant decrease in neutrophil infiltrate (Figures S3B and S3C).

To examine a cause for this reduction in leukocyte infiltrates, we looked at the expression of chemokines in the liver. We observed significant decreases in expression levels of the chemokines *CCL7* (*MCP-3*), *CCL2* (*MCP-1*), *CXCL10* (*IP-10*), *CXCL2* (*MIP-2α*), and *CXCL9* (*MIG*) in coinfected mice relative to mice infected with bacteria alone (Figure 3D). CXCL10 and CXCL9, two chemokines with proposed bactericidal activity, are both induced by IFN-γ (Cole et al., 2001); it is therefore possible that reduction in systemic IFN-γ may account for their decreased expression in the liver. CCL2 and CCL7 have both been shown to be critical chemokines in the defense against *L. monocytogenes* (Jia et al., 2008). In addition, CCL7 can be induced by IL-6; therefore, the reduction in systemic IL-6 may lead to the decrease in hepatic *CCL7* expression (Romano et al., 1997). While CCL2 recruits mainly monocytes to the site of infection, CCL7 attracts many different cell types of the immune system by binding to at least four different chemokine receptors (Menten et al., 2001). In addition to the decrease in chemokine expression, there was a decrease in the hepatic expression of the adhesion molecule *Icam-1* (Figure 3D). The reduction

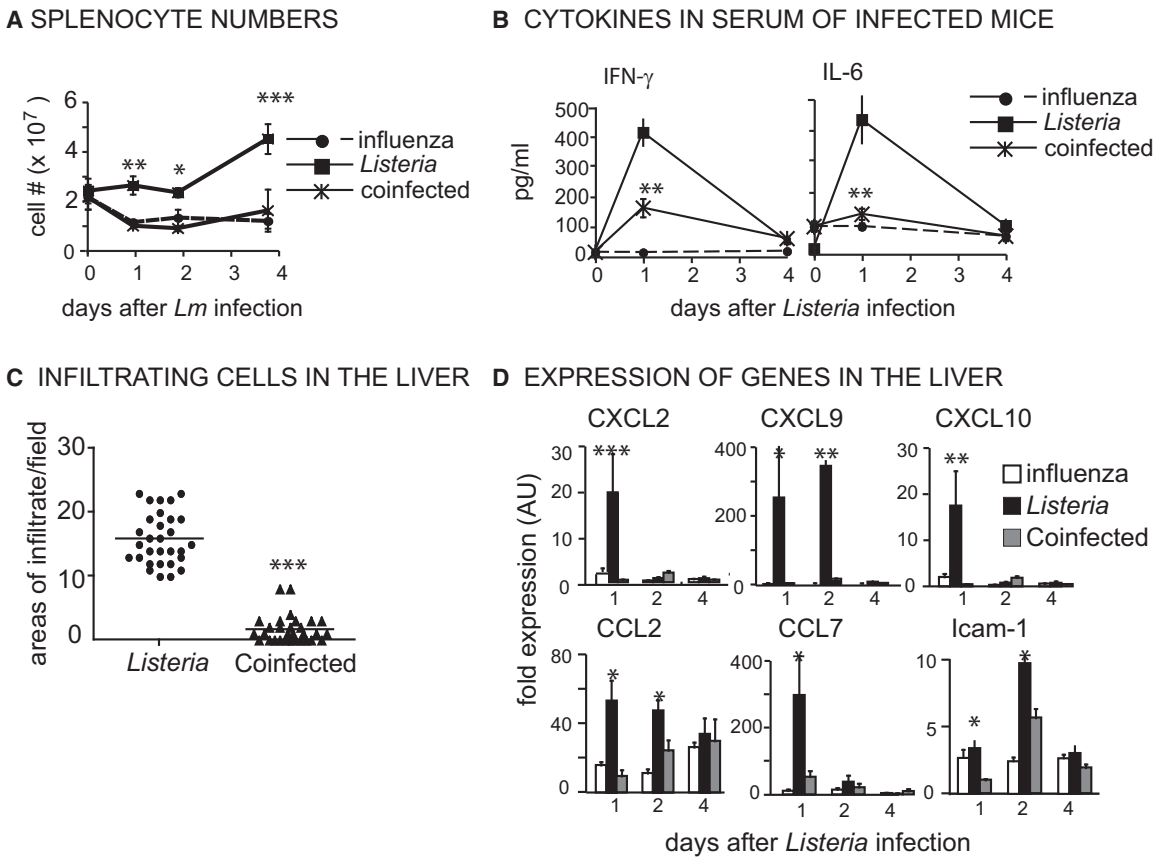

Figure 3. Influenza Virus Infection Causes Immunosuppression

(A) Splenocyte numbers were determined after infection with influenza and *L. monocytogenes*.

(B) The levels of the cytokines IFN-γ and IL-6 in the serum were measured 1 and 4 days after infection with *L. monocytogenes*. Day 0 is prior to *L. monocytogenes* infection and 1 day after influenza infection.

(C) The areas of infiltrating cells per field were determined by blind scoring of H&E-stained liver sections 4 days after infection with *L. monocytogenes*.

(D) Gene expression in the liver was determined at 1, 2, and 4 days after bacterial infection in mice infected with influenza alone, mice infected with *L. monocytogenes* alone, or coinfected mice. In all experiments, mice were infected with 10,000 pfu of influenza 1 day before infection with *L. monocytogenes*. Data are from at least three independent experiments with at least four mice in each group. The error bars represent SD (*$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$, no *$p > 0.05$). See also Figure S3.

of these critical chemokines and adhesion molecules in the liver is consistent with the observed reduction of immune cell infiltrate.

Given the delay in bacterial clearance, it seemed likely that aspects of the acquired immune response were also suppressed. Indeed, there were fewer antigen-specific $CD8^+$ T cells at day 8 after bacterial infection in mice infected 5 days previously with 200 pfu of influenza virus (Figure S2). Thus, previous infection with influenza virus suppresses many aspects of the innate and acquired immune responses to systemic bacterial infection, including production of cytokines, tissue-specific chemokines, immune cell infiltration into the infected organs, and antigen-specific T cell accumulation.

Influenza Virus-Induced GCs Cause Immunosuppression

We next examined the mechanism responsible for the suppressed immune response in coinfected mice. Levels of the immunosuppressive cytokine IL-10 were not increased in the serum of influenza-infected or coinfected mice (Figure S6A and data not shown). Although this does not formally exclude a potential contributing role of IL-10, we examined other potential mechanisms of immunosuppression. Mice infected with influenza virus showed sustained elevated levels of GCs in the serum (Figure 4A). GCs are produced by the adrenal glands in response to stress through activation of the HPA axis and are known to have multiple immunosuppressive effects (Sternberg, 2006). Inflammatory cytokines are known to induce GC, and indeed, high levels of the cytokines IL-6, IFN-γ, and IL-12 were detectable in the serum of the infected mice 24 hr after systemic infection with *L. monocytogenes* (Figure S6B). However, increased serum GC levels were only detectable in *L. monocytogenes*-infected mice at the peak of the innate immune response to infection (~24 hr), after which they quickly returned to normal levels (Figure 4A). In contrast, mice infected with influenza virus showed sustained levels of serum GCs (Figure 4A) despite the absence of high levels of systemic inflammatory cytokines (Figure S6A). Furthermore, influenza virus-induced GC levels were not decreased in *MyD88*$^{-/-}$, *IL-6*$^{-/-}$, *IL-1R*$^{-/-}$, *TNF-α*$^{-/-}$,

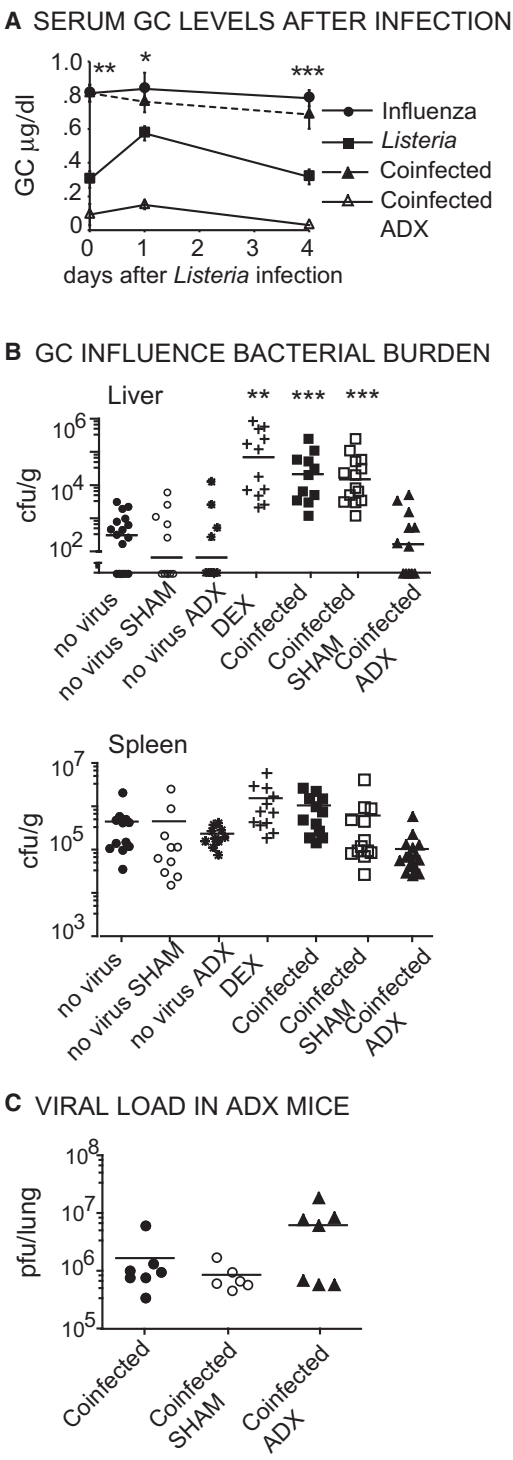

Figure 4. Influenza Virus-Induced GCs Cause Increased Bacterial Burden

(A) Serum GC levels were measured in mice infected with influenza virus, mice infected with *L. monocytogenes*, coinfected mice, and coinfected ADX mice. Day 0 indicates serum GC levels prior to bacterial infection.

(B) Bacterial cfu per gram were determined in the liver (top) and the spleen (bottom) 4 days after bacterial infection in sham-operated (SHAM) or adrenalectomized (ADX) mice infected with *L. monocytogenes* or mice coinfected with influenza and *L. monocytogenes*. In addition, cfu were measured in mice infected with *L. monocytogenes* and treated daily with DEX.

(C) Viral titers were measured in coinfected mice, coinfected SHAM mice, and coinfected ADX mice. In all experiments, mice were infected with 10,000 pfu of influenza 1 day before infection with *L. monocytogenes*. Data in (A) and (B) are from at least three independent experiments with at least four mice in each group, and data in (C) are from two independent experiments with three or four mice in each group. Bacterial cfu comparing *L. monocytogenes* alone, coinfected, and coinfected ADX was repeated in ten independent experiments with statistically significant results in each individual experiment, Mice with no detectable cfu are indicated by symbols on the x axis. The error bars represent SD (*$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$, no *$p > 0.05$). See also Figure S4.

and *IFNαR1*$^{-/-}$ mice (Figure S4). Finally, systemic administration of lipopolysaccharide (LPS) caused only modest and transient induction of GCs, while poly I:C caused no induction at all (data not shown). Collectively, these results suggest that the sustained level of serum GCs induced by influenza virus infection was independent of a systemic inflammatory response.

To examine the role of influenza infection-triggered GCs in the suppression of immune responses to a systemic bacterial infection, we took advantage of mice that had their adrenal glands surgically removed. Consistent with the requirement for adrenal glands in the production of GCs, adrenalectomized (ADX) mice did not show an increase in serum GCs in response to coinfection or influenza infection alone (Figure 4A). In addition, many of the ADX mice had levels of GCs that were below the limit of detection. Importantly, adrenalectomy reversed the effect of influenza infection on the hepatic bacterial load in coinfected mice, as the coinfected ADX mice had bacterial loads similar to mice infected with *L. monocytogenes* alone (Figure 4B). Viral titers in the lung were not statistically different between coinfected, coinfected sham-operated, and coinfected ADX (Figure 4C).

The improved clearance of bacteria in coinfected ADX mice indicated that the influenza virus infection-induced production of GCs is an important mechanism involved in suppression of the immune system during coinfection. We next analyzed the specific aspects of the innate immune response that could be the targets of GC signaling. In contrast to coinfected mice or coinfected sham-operated controls, coinfected ADX mice had higher levels of IL-6 in the serum 24 hr after *L. monocytogenes* infection (Figure 5A). The levels were similar to those seen in mice infected with *L. monocytogenes* alone, consistent with in vitro observations showing that IL-6 is regulated by GCs (Ray and Sehgal, 1992). IL-6 has also been shown to induce expression of CCL7 (Romano et al., 1997). In correlation with the observed rescue of IL-6 production, there was an increase in *CCL7* expression in the liver (Figure 5B). In addition, in ADX coinfected mice, *Icam-1* expression in the liver was also restored to nearly the same level as in mice infected with *L. monocytogenes* alone (Figure 5C). In accordance with the rescue of *CCL7* and *Icam-1* expression in the liver in ADX mice, coinfected ADX mice had levels of immune cell infiltrate similar to mice singly infected with *L. monocytogenes* (Figures 5D and S3C). Another pronounced effect of influenza virus coinfection was the decrease in spleen size. This effect was also due to GCs, as it was eliminated in coinfected ADX mice (Figure 5E). Interestingly, the effect of adrenalectomy did not extend to all proinflammatory signaling pathways, as serum levels of IFN-γ were not affected

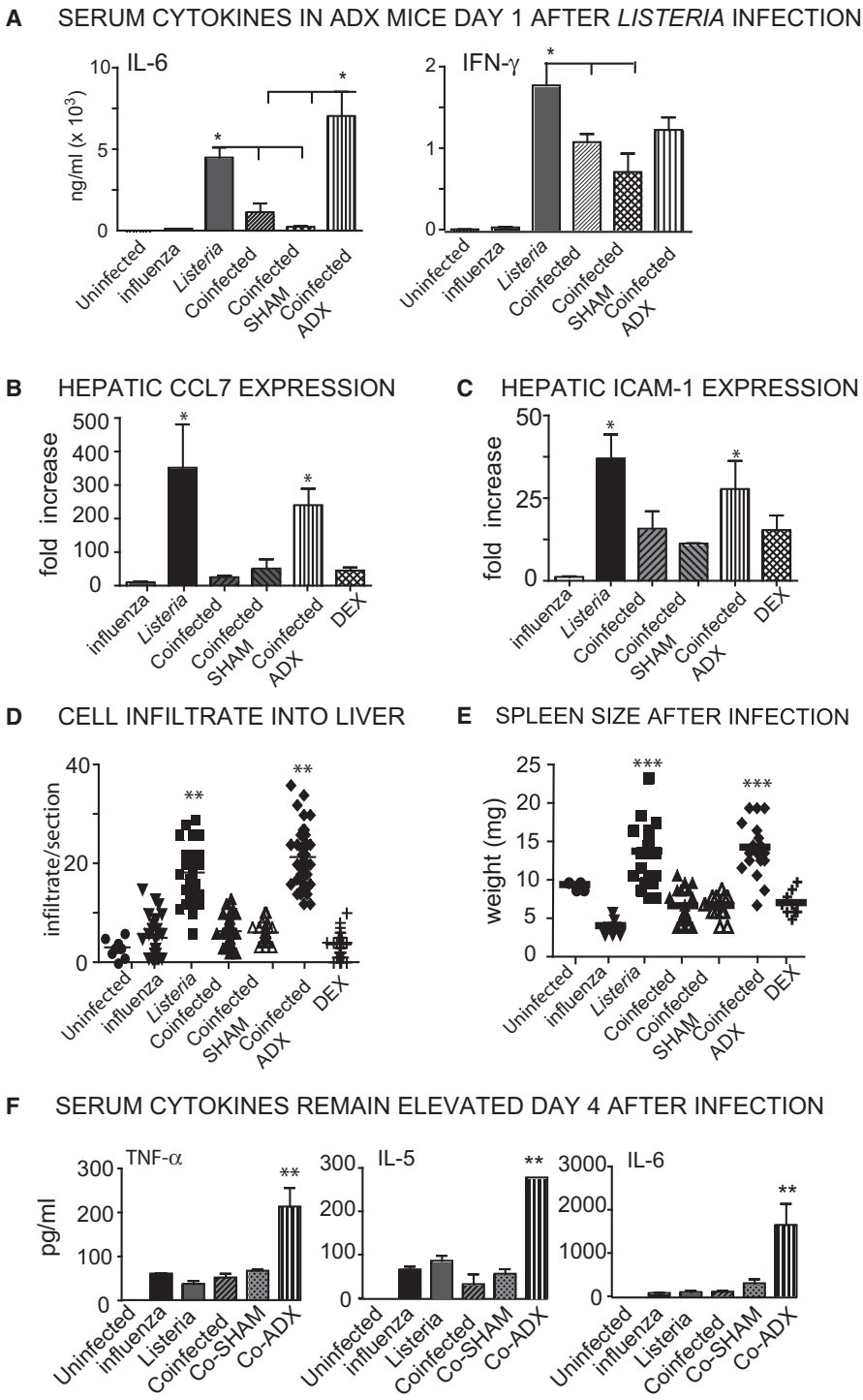

Figure 5. Removal of GC Rescues Virus-Induced Immunosuppression

(A–C) Serum levels of IL-6 and IFN-γ and hepatic expression of *CCL7* (B) and *Icam-1* (C) were determined 1 day after infection with bacteria in mice infected with influenza virus, mice infected with *L. monocytogenes*, coinfected mice, coinfected SHAM mice, coinfected ADX mice, and *L. monocytogenes*-infected mice treated with DEX.

(D and E) The amount of immune cell infiltrate into the liver (D) and spleen weight (E) were determined 4 days after bacterial infection in uninfected mice, mice infected with single pathogens, coinfected mice, coinfected SHAM mice, coinfected ADX mice, and *L. monocytogenes*-infected mice treated with DEX.

(F) The cytokines TNF-α, IL-5, and IL-6 were measured in the serum 4 days after infection with *L. monocytogenes*, in mice that were infected with influenza and *L. monocytogenes*, coinfected ADX mice, coinfected SHAM mice, and singly infected controls. Data are from at least three independent experiments with at least four mice in each group. The error bars represent SD (*$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$, no *$p > 0.05$). See also Figure S5.

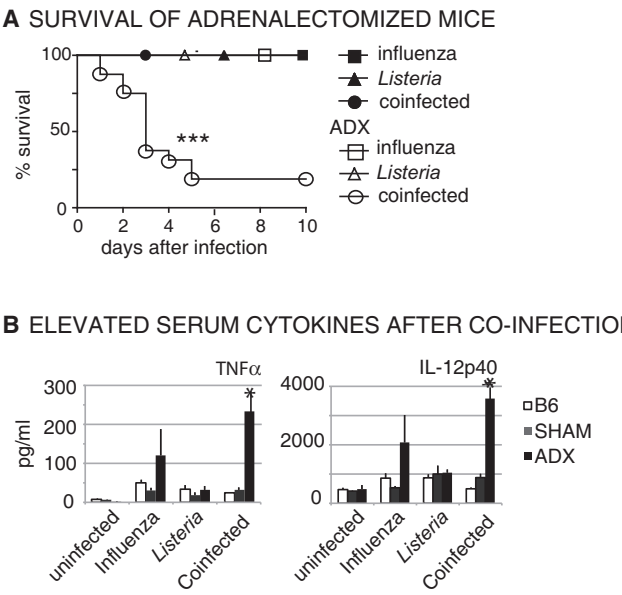

Figure 6. ADX Mice Succumb to Coinfection

(A) ADX mice infected with a sublethal dose of influenza virus (200 pfu) and 5 days later a sublethal dose of *L. monocytogenes* (5000 cfu) had decreased survival compared to singly infected ADX mice, singly infected mice, or coinfected mice.

(B) The cytokines TNF-α and IL-12p40 were elevated in the serum of coinfected ADX mice as early as 1 day after bacterial infection. Data are from at least two independent experiments with at least four mice in each group. The error bars represent SD (*$p \leq 0.05$, ***$p \leq 0.0001$, no *$p > 0.05$). See also Figure S6.

by adrenalectomy, in contrast to IL-6 (Figure 5A). Accordingly, IFN-γ induced chemokines also were not rescued in coinfected ADX mice (data not shown). However, given the rescue of the bacterial burden in ADX mice, the levels of these chemokines and cytokines must be sufficient.

Further corroborating the hypothesis that virus-induced GCs were responsible for the increased bacterial load, mice treated daily with the synthetic corticosteroid dexamethasone (DEX) starting 1 day prior to infection with *L. monocytogenes* also showed increased bacterial loads in the liver (Figure 4B). Also, the immunosuppression by influenza-induced GCs was mimicked by DEX treatment. *CCL7* and *Icam-1* expression in the liver were decreased in mice treated with DEX, and there was a corresponding decrease in cellular infiltrates (Figures 5B–5D). In addition, the spleens of *L. monocytogenes*-infected/DEX-treated mice showed a decrease in size (Figure 5E). Importantly, the DEX levels used in these experiments were determined by a bioassay to be physiologically similar to the levels of GCs measured during influenza infection (Figure S5).

These results show that GC production triggered by an infection with influenza virus led to a suppression of key aspects of the innate immune response to *L. monocytogenes*, including the production of IL-6 and the hepatic expression of *CCL7* and *Icam-1*. Suppression of these key factors of the innate immune response resulted in an increased bacterial burden. Blockade of GC production prevented the suppression of the innate immune response and reduced the early bacterial load in the liver. In addition, treatment with DEX mimicked many of the aspects of immunosuppression of the innate immune response to *L. monocytogenes* caused by infection with influenza virus.

ADX Coinfected Mice Have an Increased Inflammatory Response and Decreased Survival

While many aspects of the influenza-induced immunosuppression, including the defect in bacterial clearance, were rescued in ADX mice, prevention of GC production is not universally beneficial to the host. Indeed, GCs are known to play an important role in controlling excessive inflammation. Accordingly, coinfected ADX mice had large increases in the systemic inflammatory cytokines, IL-6, IL-5, and TNF-α, 4 days after infection with *L. monocytogenes* and 5 days after infection with influenza virus (Figure 5F). To examine the effect of the increased inflammatory response further, mice were infected with a sublethal dose of influenza virus, followed 5 days later by a sublethal dose of *L. monocytogenes*. While all non-ADX mice survived coinfection, the majority of the coinfected ADX mice did not survive past 5 days after the bacterial infection, and many started to succumb as early as 1 day after bacterial infection (Figure 6A). In contrast, both ADX and non-ADX mice infected with a sublethal dose of either *L. monocytogenes* (5000 cfu) or influenza virus (200 pfu) alone were able to survive for up to 30 days after infection (Figure 6A and data not shown).

When we further examined this sudden decrease in survival of the coinfected ADX mice, we found that there was a systemic increase in two key inflammatory cytokines. There was a large increase in serum levels of TNF-α and IL-12p40 1 day after infection with *L. monocytogenes* in coinfected ADX mice, as compared to the singly infected controls (Figure 6B). Both TNF-α and IL-12p40 are known to be suppressed by GCs (Rogatsky and Ivashkiv, 2006).

Since our coinfected mice were infected with influenza virus for 5 days before infection with *L. monocytogenes*, it seemed likely that there would also be significant alterations specifically in the pulmonary immune response. There were large increases in a number of inflammatory cytokines in the bronchoalveolar lavage fluid (BALF) (Figure 7A). IL-6, IL-12p40, TNF-α, and IFN-γ were all increased in coinfected ADX mice to a greater extent than any other group (Figure 7A). IL-1β and IL-5 were increased in influenza-infected ADX mice and coinfected ADX mice (Figure 7A). These cytokines are all known to be subject to control by GCs (Besedovsky et al., 1986; Ray and Sehgal, 1992). In addition, there was a dramatic increase in immunopathology noted in the lungs of influenza-infected and coinfected ADX mice (Figures 7B and S7). There was also an increased level of albumin in the BALF of coinfected ADX mice, which suggests increased epithelial tissue damage (Figure 7C). In contrast, there were no detectable changes in the liver determined by histological analysis and expression of inflammatory genes (data not shown).

Thus, while the production of GCs triggered by influenza virus infection transiently suppressed the immune response to a secondary bacterial infection, it was necessary to control the inflammatory response during coinfection in order to prevent lethal immunopathology.

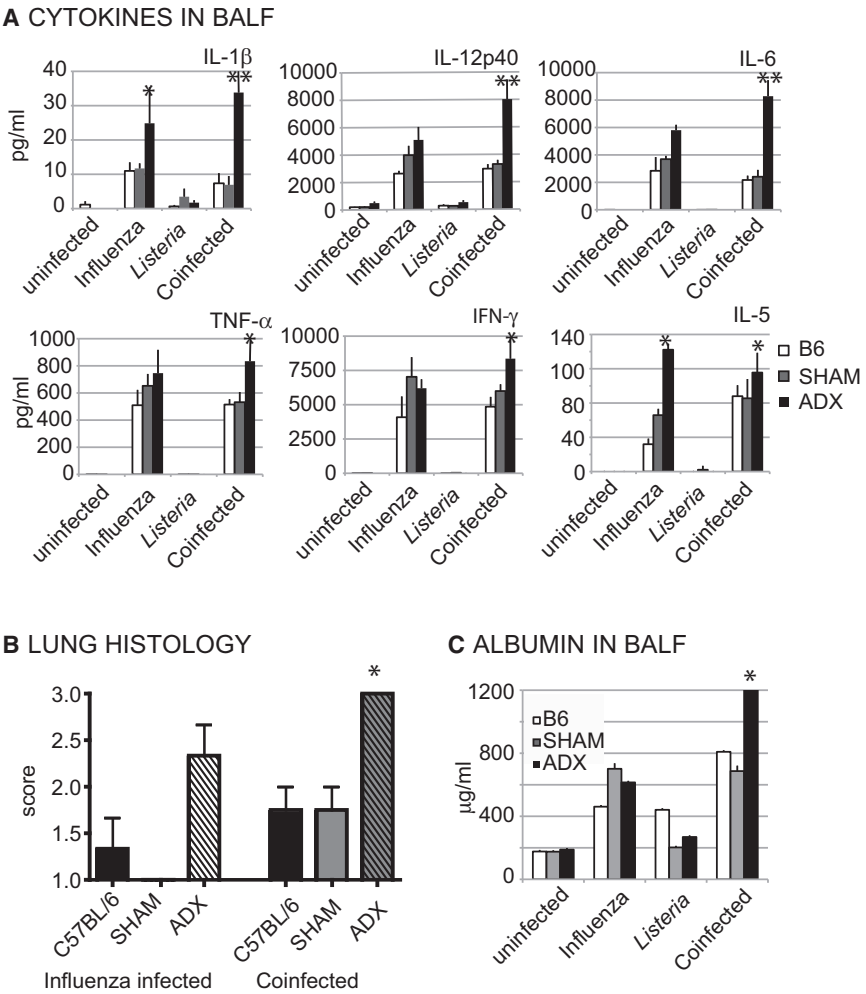

Figure 7. Lack of GC Causes Increased Inflammation and Immunopathology

(A) Coinfected ADX mice have increased levels of IL-1β, IL-12p40, IL-6, IL-5, TNF-α, and IFN-γ in the bronchoalveolar lavage fluid (BALF).

(B) Coinfected ADX mice had increased lung pathology.

(C) As a marker for epithelial cell damage, albumin was measured in the BALF, and this was also increased in coinfected ADX mice. Data are from at least two independent experiments with at least four mice in each group. The error bars represent SD (*$p \leq 0.05$, **$p \leq 0.001$, no *$p > 0.05$). See also Figure S7.

DISCUSSION

The immune system undergoes a series of dramatic changes during a response to infection. Exposure to a secondary infection while the host undergoes these alterations may affect host defense capabilities in multiple ways, depending on the types of infections. Influenza virus infection is known to increase susceptibility to secondary bacterial infections, particularly opportunistic bacterial pathogens of the respiratory tract (Beadling and Slifka, 2004). Many studies have focused on mechanisms of influenza virus-mediated immunosuppression in the lung (Didierlaurent et al., 2008; Shahangian et al., 2009; Sun and Metzger, 2008; van der Sluijs et al., 2004). However, the effect of influenza virus infection on systemic immunity is less well understood. Many bacterial pathogens that initially infect the lung will subsequently become systemic, and it is therefore important to understand the impact of influenza virus on the immune response to a systemic bacterial pathogen. Here, we investigated how respiratory influenza infection affects the systemic innate immune response. We chose the model bacterial pathogen *L. monocytogenes* to investigate the effects of influenza infection on subsequent systemic bacterial infection, because the innate and adaptive immune responses to this pathogen are well characterized (Pamer, 2004; Unanue, 1997a). Using this model system, we found that influenza infection caused a profound systemic immunosuppression, as indicated by a blunted response to systemic infection with *Listeria*. Specifically, we found decreased production of inflammatory cytokines and chemokines, reduced recruitment of innate immune cells to the infection site (liver), and, as a result, a dramatic increase in bacterial burden. Surprisingly, this systemic immunosuppressive effect was mediated by a sustained production of GCs triggered by the influenza infection. Surgical removal of the adrenal glands, which are the main source of GCs, eliminated most of the immunosuppressive effects of influenza infection. Accordingly, ADX mice were able to better suppress secondary bacterial infection. Interestingly, ADX mice had a relatively normal immune response to single viral or bacterial infections. However, the lack of the GC response in coinfected mice, while allowing for an improved antibacterial immunity, eventually resulted in an exacerbated inflammatory response, leading to lethality.

GCs are crucial for the homeostatic regulation of many physiological systems (Tasker, 2006; Viegas et al., 2008). In the immune response, they function to regulate inflammation and maintain immune homeostasis (Di Comite et al., 2007; Sternberg, 2006). GCs are produced by adrenal glands through activation of the HPA axis by different types of psychological and physiological stress (Di Comite et al., 2007; Rogatsky and Ivashkiv, 2006; Ruzek et al., 1999). One type of physiological stress that activates the HPA axis is excessive production of inflammatory cytokines during systemic inflammatory response syndrome (SIRS) (Beishuizen and Thijs, 2003; Webster and Sternberg, 2004). Many bacterial and viral infections have been shown to trigger the HPA axis because of an increase in systemic inflammatory cytokines, and often the resulting GCs are necessary to prevent an excessive inflammatory response (Besedovsky et al., 1986; Besedovsky and del Rey, 1989; Dunn and Vickers, 1994; Hermann et al., 1994; Ruzek et al., 1997, 1999). In this

study, we demonstrate that infection with influenza virus induces a sustained production of GCs in the absence of systemic inflammation. In addition, we show that in mice deficient in the classic inflammatory components that are known to induce GCs (Silverman et al., 2005), influenza virus infection still induces GC production, while a potent inflammatory response induced by systemic administration of LPS or poly I:C did not result in the same magnitude or duration of GC production. Collectively, these data suggest that lung infection with influenza virus induces GCs through a pathway that is at least in part independent of systemic inflammatory cytokines. Influenza virus causes lung damage both directly, through viral infection of epithelial cells, and indirectly, through induction of the inflammatory response (La Gruta et al., 2007). Damage to a crucial organ, such as the lung, causes a generalized stress response (Mendez and Hubmayr, 2005), which can trigger GC production. We therefore suggest that lung tissue damage caused by influenza virus infection triggers the HPA response and GC production. Tissue damage in this case may be perceived through the group C nerve fibers, which play an essential role in monitoring tissue homeostasis (Craig, 2002). However, the exact mechanism linking infection-induced tissue damage to the HPA axis activation is currently unknown.

The prolonged elevated GC levels triggered by influenza virus infection affected several key aspects of the innate immune response. Here, we show that the induction of the essential cytokine IL-6 in response to *L. monocytogenes* infection was suppressed by influenza virus-induced GCs. Several key aspects of the hepatic immune response to bacterial infection (Jia et al., 2008; López et al., 1999) were also suppressed during coinfection, including the chemokine *CCL7* and the adhesion molecule *Icam-1*. Correlating with the decrease in hepatic chemokine and adhesion molecule expression, there was a significant decrease in infiltrating immune cells into the liver in coinfected mice.

Corroborating the data with virus-induced GCs, daily treatment with the synthetic corticosteroid DEX also affected the innate immune response in the liver. Mice treated daily with DEX had increased bacterial cfu in the liver comparable to mice infected with influenza virus. The specific aspects of the antibacterial innate immune response that were suppressed in influenza-infected mice and rescued in ADX mice were also suppressed in mice treated with DEX. Mice pretreated with DEX and infected with *L. monocytogenes* had decreased expression of the chemokine *CCL7*, decreased expression of the adhesion molecule *Icam-1*, and subsequent decreased cellular infiltration into the liver.

We observed that the liver was the major target organ of early immunosuppression and the site of increased bacterial burden in response to influenza-induced endogenous GCs or exogenous DEX. There are several factors that may contribute to this organ-specific suppression. The liver is a very important organ in the immune response to many bacterial infections of the blood and is known specifically to be important in the innate immune response to *L. monocytogenes* (Cousens and Wing, 2000; Ebe et al., 1999; López et al., 1999). Regulation of the immune response by IL-6 is very important for the immune response in the liver, and mice deficient in IL-6 have a greater defect in the immune response of the liver than the spleen (Dalrymple et al., 1995). The liver has a high expression of GC receptors, and this expression can be upregulated during times of stress (Dong et al., 1988; Turner et al., 2006). Also, in the liver, hepatocytes as well as macrophages support *L. monocytogenes* growth, while in the spleen, growth occurs mainly in macrophages. The disappearance of these niche cells in the spleen due to GC-induced apoptosis is likely to offset the immunosuppressive effect. A combination of these factors could account for the influenza virus-induced increase in bacterial load specifically in the liver.

In this study, we also found that GCs are necessary to control excessive inflammation during coinfection and prevent a lethal immunopathology. ADX mice infected with sublethal levels of influenza virus followed 5 days later by a sublethal dose of *L. monocytogenes* succumb within days of the bacterial infection. These mice have some signs of slightly increased systemic inflammation, such as elevated levels of the cytokines TNF-α and IL-12p40 in the serum; however, the most dramatic effects of coinfection were observed mainly in the lung. Many inflammatory cytokines in the BALF are dramatically increased in coinfected ADX mice. Consequently, the lungs of coinfected ADX mice had increased signs of immunopathology, including excess immune cell infiltrates and epithelial cell necrosis. ADX mice infected with influenza virus alone also have increased inflammatory responses in the lung, but these responses are further increased in the coinfected ADX mice.

Bacterial complications from viral infections cause more morbidity and mortality than viral infections alone (Beadling and Slifka, 2004). We have delineated a mechanism by which infection with influenza virus, through the induction of GCs, leads to suppression of the systemic immune response to a secondary bacterial infection and therefore an increased bacterial burden. However, we also found that the induction of GCs is critical for survival of coinfection. When infected with influenza virus and *L. monocytogenes*, mice lacking the ability to produce GCs had a greatly exacerbated inflammatory response, particularly in the lung, and did not survive coinfection. Collectively, our findings reveal the inflammation-independent activation of the HPA axis by influenza virus infection and demonstrate its physiological and pathological consequences for a systemic bacterial coinfection.

EXPERIMENTAL PROCEDURES

Mice

C57BL/6 (B6) mice were obtained from the National Cancer Institute and the Jackson Laboratory and were between 6 and 12 weeks of age. ADX mice were obtained from Taconic Laboratories (Germantown, NY). All other mice were bred in the Yale University School of Medicine animal facility in specific pathogen-free conditions. GC levels in the serum were measured by the animal healthcare service Antech. DEX was administered intraperitoneally at a daily dose of 1 mg per mouse starting 1 day before infection. All experiments were performed in accordance with the institutional animal care and use guidelines.

Bacterial and Viral Strains

The A/WSN/33 (H1N1) strain of influenza virus was obtained from the laboratory of Akiko Iwasaki and propagated using MDCK cells as described in Okuda et al. (2001). The 10403S *L. monocytogenes* strain used was propagated in BHI agar as described in Auerbuch et al. (2004). Influenza virus was administered intranasally at the indicated doses in a volume of 30 μl. Mice were infected with *L. monocytogenes* intravenously at the indicated doses in a volume of 100 μl.

Quantification of Bacterial and Viral Loads

Viral titers in the lungs were determined by titration of organ homogenates on MDCK cells as described in Okuda et al. (2001). *L. monocytogenes* cfu were determined by plating titrating doses of organ homogenate on agar plates as previously described (Auerbuch et al., 2004).

Measurement of Cytokines and Chemokines

Briefly, tissues were disrupted in RNA-bee (Teletest; Friendswood, TX), and RNA was isolated according to the manufacturer's instructions. After treatment with DNase, SuperScript III was used to produce cDNA. Quantitative PCR was done on Eppendorf Mastercycler with QIAGEN SYBR Green mix according to the manufacturer's instructions. Serum cytokine levels were measured using the Luminex system according to the manufacturer's instructions.

Tissue Histology

Organs used for histology were fixed overnight in Formalde-Fresh and embedded in paraffin for sectioning. Sections were stained with H&E according to the manufacturer's instructions and blind-randomized scored. For each liver section, 40 pictures were taken, and the areas of infiltrate were counted.

Statistical analysis was performed using the Prism graphing and analysis software. Comparison of multiple groups was done using ANOVA, and comparison of two groups was done with the Student's t test.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and can be found with this article online at doi:10.1016/j.chom.2010.01.010.

ACKNOWLEDGMENTS

The authors would like to thank Sophie Holley for technical assistance; Gergana Galabova-Kovacs for advice on histological analysis; Takeshi Ichinohe, Melissa Lineham, and Akiko Iwasaki for viral strains and advice; and Noah Palm and Igor Brodsky for critical reading of the manuscript. This work was supported by the Ellison Foundation, the Howard Hughes Medical Institute, and the NIH (AI055502, AI046688, and DK071754). A.M.J. is a Berger Foundation Fellow of the Damon Runyon Cancer Research Foundation.

Received: September 3, 2009
Revised: January 2, 2010
Accepted: January 27, 2010
Published: February 17, 2010

REFERENCES

Andersson, A., Dai, W.J., Di Santo, J.P., and Brombacher, F. (1998). Early IFN-gamma production and innate immunity during Listeria monocytogenes infection in the absence of NK cells. J. Immunol. *161*, 5600–5606.

Auerbuch, V., Brockstedt, D.G., Meyer-Morse, N., O'Riordan, M., and Portnoy, D.A. (2004). Mice lacking the type I interferon receptor are resistant to Listeria monocytogenes. J. Exp. Med. *200*, 527–533.

Bakaletz, L.O. (2004). Developing animal models for polymicrobial diseases. Nat. Rev. Microbiol. *2*, 552–568.

Barton, E.S., White, D.W., Cathelyn, J.S., Brett-McClellan, K.A., Engle, M., Diamond, M.S., Miller, V.L., and Virgin, H.W., 4th. (2007). Herpesvirus latency confers symbiotic protection from bacterial infection. Nature *447*, 326–329.

Beadling, C., and Slifka, M.K. (2004). How do viral infections predispose patients to bacterial infections? Curr. Opin. Infect. Dis. *17*, 185–191.

Beishuizen, A., and Thijs, L.G. (2003). Endotoxin and the hypothalamo-pituitary-adrenal (HPA) axis. J. Endotoxin Res. *9*, 3–24.

Berendt, R.F., and Jaax, N.K. (1985). Effect of prior influenza virus infections on susceptibility of AKR/J mice to respiratory challenge with Legionella pneumophila. J. Lab. Clin. Med. *105*, 124–131.

Besedovsky, H.O., and del Rey, A. (1989). Mechanism of virus-induced stimulation of the hypothalamus-pituitary-adrenal axis. J. Steroid Biochem. *34*, 235–239.

Besedovsky, H., del Rey, A., Sorkin, E., and Dinarello, C.A. (1986). Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. Science *233*, 652–654.

Bethin, K.E., Vogt, S.K., and Muglia, L.J. (2000). Interleukin-6 is an essential, corticotropin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. Proc. Natl. Acad. Sci. USA *97*, 9317–9322.

Borrow, P., Hou, S., Gloster, S., Ashton, M., and Hyland, L. (2005). Virus infection-associated bone marrow B cell depletion and impairment of humoral immunity to heterologous infection mediated by TNF-alpha/LTalpha. Eur. J. Immunol. *35*, 524–532.

Carrero, J.A., Calderon, B., and Unanue, E.R. (2004). Type I interferon sensitizes lymphocytes to apoptosis and reduces resistance to Listeria infection. J. Exp. Med. *200*, 535–540.

Cole, A.M., Ganz, T., Liese, A.M., Burdick, M.D., Liu, L., and Strieter, R.M. (2001). Cutting edge: IFN-inducible ELR- CXC chemokines display defensin-like antimicrobial activity. J. Immunol. *167*, 623–627.

Cousens, L.P., and Wing, E.J. (2000). Innate defenses in the liver during Listeria infection. Immunol. Rev. *174*, 150–159.

Craig, A.D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. Nat. Rev. Neurosci. *3*, 655–666.

Dai, W.J., Bartens, W., Köhler, G., Hufnagel, M., Kopf, M., and Brombacher, F. (1997). Impaired macrophage listericidal and cytokine activities are responsible for the rapid death of Listeria monocytogenes-infected IFN-gamma receptor-deficient mice. J. Immunol. *158*, 5297–5304.

Dalrymple, S.A., Lucian, L.A., Slattery, R., McNeil, T., Aud, D.M., Fuchino, S., Lee, F., and Murray, R. (1995). Interleukin-6-deficient mice are highly susceptible to Listeria monocytogenes infection: correlation with inefficient neutrophilia. Infect. Immun. *63*, 2262–2268.

Decker, T., Müller, M., and Stockinger, S. (2005). The yin and yang of type I interferon activity in bacterial infection. Nat. Rev. Immunol. *5*, 675–687.

Di Comite, G., Grazia Sabbadini, M., Corti, A., Rovere-Querini, P., and Manfredi, A.A. (2007). Conversation galante: how the immune and the neuroendocrine systems talk to each other. Autoimmun. Rev. *7*, 23–29.

Didierlaurent, A., Goulding, J., Patel, S., Snelgrove, R., Low, L., Bebien, M., Lawrence, T., van Rijt, L.S., Lambrecht, B.N., Sirard, J.C., and Hussell, T. (2008). Sustained desensitization to bacterial Toll-like receptor ligands after resolution of respiratory influenza infection. J. Exp. Med. *205*, 323–329.

Dong, Y., Poellinger, L., Gustafsson, J.A., and Okret, S. (1988). Regulation of glucocorticoid receptor expression: evidence for transcriptional and post-translational mechanisms. Mol. Endocrinol. *2*, 1256–1264.

Dunn, A.J., and Vickers, S.L. (1994). Neurochemical and neuroendocrine responses to Newcastle disease virus administration in mice. Brain Res. *645*, 103–112.

Dunn, A.J., Powell, M.L., Meitin, C., and Small, P.A., Jr. (1989). Virus infection as a stressor: influenza virus elevates plasma concentrations of corticosterone, and brain concentrations of MHPG and tryptophan. Physiol. Behav. *45*, 591–594.

Ebe, Y., Hasegawa, G., Takatsuka, H., Umezu, H., Mitsuyama, M., Arakawa, M., Mukaida, N., and Naito, M. (1999). The role of Kupffer cells and regulation of neutrophil migration into the liver by macrophage inflammatory protein-2 in primary listeriosis in mice. Pathol. Int. *49*, 519–532.

Elston, J.W., and Thaker, H.K. (2008). Coinfection with human immunodeficiency virus and tuberculosis. Indian J. Dermatol. Venereol. Leprol. *74*, 194–199.

García-Sastre, A., Durbin, R.K., Zheng, H., Palese, P., Gertner, R., Levy, D.E., and Durbin, J.E. (1998). The role of interferon in influenza virus tissue tropism. J. Virol. *72*, 8550–8558.

Gardner, I.D. (1981). Suppression of antibacterial immunity by infection with influenza virus. J. Infect. Dis. *144*, 225–231.

Gregory, S.H., and Wing, E.J. (2002). Neutrophil-Kupffer cell interaction: a critical component of host defenses to systemic bacterial infections. J. Leukoc. Biol. *72*, 239–248.

Gumenscheimer, M., Balkow, S., Simon, M.M., Jirillo, E., Galanos, C., and Freudenberg, M.A. (2007). Stage of primary infection with lymphocytic choriomeningitis virus determines predisposition or resistance of mice to secondary bacterial infections. Med. Microbiol. Immunol. (Berl.) *196*, 79–88.

Hament, J.M., Kimpen, J.L., Fleer, A., and Wolfs, T.F. (1999). Respiratory viral infection predisposing for bacterial disease: a concise review. FEMS Immunol. Med. Microbiol. *26*, 189–195.

Hermann, G., Tovar, C.A., Beck, F.M., and Sheridan, J.F. (1994). Kinetics of glucocorticoid response to restraint stress and/or experimental influenza viral infection in two inbred strains of mice. J. Neuroimmunol. *49*, 25–33.

Humphreys, T.D., Khanolkar, A., Badovinac, V.P., and Harty, J.T. (2008). Generation and maintenance of Listeria-specific CD8+ T cell responses in perforin-deficient mice chronically infected with LCMV. Virology *370*, 310–322.

Jia, T., Serbina, N.V., Brandl, K., Zhong, M.X., Leiner, I.M., Charo, I.F., and Pamer, E.G. (2008). Additive roles for MCP-1 and MCP-3 in CCR2-mediated recruitment of inflammatory monocytes during Listeria monocytogenes infection. J. Immunol. *180*, 6846–6853.

Julkunen, I., Sareneva, T., Pirhonen, J., Ronni, T., Melén, K., and Matikainen, S. (2001). Molecular pathogenesis of influenza A virus infection and virus-induced regulation of cytokine gene expression. Cytokine Growth Factor Rev. *12*, 171–180.

Kaufmann, S.H. (1993). Immunity to intracellular bacteria. Annu. Rev. Immunol. *11*, 129–163.

Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., Kishimoto, T., Zinkernagel, R., Bluethmann, H., and Köhler, G. (1994). Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature *368*, 339–342.

La Gruta, N.L., Kedzierska, K., Stambas, J., and Doherty, P.C. (2007). A question of self-preservation: immunopathology in influenza virus infection. Immunol. Cell Biol. *85*, 85–92.

López, S., Prats, N., and Marco, A.J. (1999). Expression of E-selectin, P-selectin, and intercellular adhesion molecule-1 during experimental murine listeriosis. Am. J. Pathol. *155*, 1391–1397.

Marcus, P.I., Rojek, J.M., and Sekellick, M.J. (2005). Interferon induction and/or production and its suppression by influenza A viruses. J. Virol. *79*, 2880–2890.

McCullers, J.A. (2006). Insights into the interaction between influenza virus and pneumococcus. Clin. Microbiol. Rev. *19*, 571–582.

Mendez, J.L., and Hubmayr, R.D. (2005). New insights into the pathology of acute respiratory failure. Curr. Opin. Crit. Care *11*, 29–36.

Menten, P., Wuyts, A., and Van Damme, J. (2001). Monocyte chemotactic protein-3. Eur. Cytokine Netw. *12*, 554–560.

Navarini, A.A., Recher, M., Lang, K.S., Georgiev, P., Meury, S., Bergthaler, A., Flatz, L., Bille, J., Landmann, R., Odermatt, B., et al. (2006). Increased susceptibility to bacterial superinfection as a consequence of innate antiviral responses. Proc. Natl. Acad. Sci. USA *103*, 15535–15539.

Noone, C.M., Lewis, E.A., Frawely, A.B., Newman, R.W., Mahon, B.P., Mills, K.H., and Johnson, P.A. (2005). Novel mechanism of immunosuppression by influenza virus haemagglutinin: selective suppression of interleukin 12 p35 transcription in murine bone marrow-derived dendritic cells. J. Gen. Virol. *86*, 1885–1890.

O'Connell, R.M., Saha, S.K., Vaidya, S.A., Bruhn, K.W., Miranda, G.A., Zarnegar, B., Perry, A.K., Nguyen, B.O., Lane, T.F., Taniguchi, T., et al. (2004). Type I interferon production enhances susceptibility to Listeria monocytogenes infection. J. Exp. Med. *200*, 437–445.

Okuda, K., Ihata, A., Watabe, S., Okada, E., Yamakawa, T., Hamajima, K., Yang, J., Ishii, N., Nakazawa, M., Okuda, K., et al. (2001). Protective immunity against influenza A virus induced by immunization with DNA plasmid containing influenza M gene. Vaccine *19*, 3681–3691.

Pamer, E.G. (2004). Immune responses to Listeria monocytogenes. Nat. Rev. Immunol. *4*, 812–823.

Peltola, V.T., and McCullers, J.A. (2004). Respiratory viruses predisposing to bacterial infections: role of neuraminidase. Pediatr. Infect. Dis. J. ***23***(1, Suppl), S87–S97.

Portnoy, D.A., Auerbuch, V., and Glomski, I.J. (2002). The cell biology of Listeria monocytogenes infection: the intersection of bacterial pathogenesis and cell-mediated immunity. J. Cell Biol. *158*, 409–414.

Ray, A., and Sehgal, P.B. (1992). Cytokines and their receptors: molecular mechanism of interleukin-6 gene repression by glucocorticoids. J. Am. Soc. Nephrol. ***2***(12, Suppl), S214–S221.

Rénia, L., and Potter, S.M. (2006). Coinfection of malaria with HIV: an immunological perspective. Parasite Immunol. *28*, 589–595.

Rogatsky, I., and Ivashkiv, L.B. (2006). Glucocorticoid modulation of cytokine signaling. Tissue Antigens *68*, 1–12.

Romano, M., Sironi, M., Toniatti, C., Polentarutti, N., Fruscella, P., Ghezzi, P., Faggioni, R., Luini, W., van Hinsbergh, V., Sozzani, S., et al. (1997). Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. Immunity *6*, 315–325.

Ruzek, M.C., Miller, A.H., Opal, S.M., Pearce, B.D., and Biron, C.A. (1997). Characterization of early cytokine responses and an interleukin (IL)-6-dependent pathway of endogenous glucocorticoid induction during murine cytomegalovirus infection. J. Exp. Med. *185*, 1185–1192.

Ruzek, M.C., Pearce, B.D., Miller, A.H., and Biron, C.A. (1999). Endogenous glucocorticoids protect against cytokine-mediated lethality during viral infection. J. Immunol. *162*, 3527–3533.

Salmi, M., Adams, D., and Jalkanen, S. (1998). Cell adhesion and migration. IV. Lymphocyte trafficking in the intestine and liver. Am. J. Physiol. *274*, G1–G6.

Seki, M., Higashiyama, Y., Tomono, K., Yanagihara, K., Ohno, H., Kaneko, Y., Izumikawa, K., Miyazaki, Y., Hirakata, Y., Mizuta, Y., et al. (2004). Acute infection with influenza virus enhances susceptibility to fatal pneumonia following Streptococcus pneumoniae infection in mice with chronic pulmonary colonization with Pseudomonas aeruginosa. Clin. Exp. Immunol. *137*, 35–40.

Selmi, C., Mackay, I.R., and Gershwin, M.E. (2007). The immunological milieu of the liver. Semin. Liver Dis. *27*, 129–139.

Shahangian, A., Chow, E.K., Tian, X., Kang, J.R., Ghaffari, A., Liu, S.Y., Belperio, J.A., Cheng, G., and Deng, J.C. (2009). Type I IFNs mediate development of postinfluenza bacterial pneumonia in mice. J. Clin. Invest. *119*, 1910–1920.

Silverman, M.N., Pearce, B.D., Biron, C.A., and Miller, A.H. (2005). Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. Viral Immunol. *18*, 41–78.

Sternberg, E.M. (2006). Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. Nat. Rev. Immunol. *6*, 318–328.

Sun, K., and Metzger, D.W. (2008). Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection. Nat. Med. *14*, 558–564.

Tasker, J.G. (2006). Rapid glucocorticoid actions in the hypothalamus as a mechanism of homeostatic integration. Obesity (Silver Spring) *14* (*Suppl 5*), 259S–265S.

Tumpey, T.M., Lu, X., Morken, T., Zaki, S.R., and Katz, J.M. (2000). Depletion of lymphocytes and diminished cytokine production in mice infected with a highly virulent influenza A (H5N1) virus isolated from humans. J. Virol. *74*, 6105–6116.

Turner, J.D., Schote, A.B., Macedo, J.A., Pelascini, L.P., and Muller, C.P. (2006). Tissue specific glucocorticoid receptor expression, a role for alternative first exon usage? Biochem. Pharmacol. *72*, 1529–1537.

Unanue, E.R. (1997a). Inter-relationship among macrophages, natural killer cells and neutrophils in early stages of Listeria resistance. Curr. Opin. Immunol. *9*, 35–43.

Unanue, E.R. (1997b). Studies in listeriosis show the strong symbiosis between the innate cellular system and the T-cell response. Immunol. Rev. *158*, 11–25.

van der Sluijs, K.F., van Elden, L.J., Nijhuis, M., Schuurman, R., Pater, J.M., Florquin, S., Goldman, M., Jansen, H.M., Lutter, R., and van der Poll, T.

(2004). IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection. J. Immunol. *172*, 7603–7609.

Viegas, L.R., Hoijman, E., Beato, M., and Pecci, A. (2008). Mechanisms involved in tissue-specific apopotosis regulated by glucocorticoids. J. Steroid Biochem. Mol. Biol. *109*, 273–278.

Wang, X., Li, M., Zheng, H., Muster, T., Palese, P., Beg, A.A., and García-Sastre, A. (2000). Influenza A virus NS1 protein prevents activation of NF-kappaB and induction of alpha/beta interferon. J. Virol. *74*, 11566–11573.

Webster, J.I., and Sternberg, E.M. (2004). Role of the hypothalamic-pituitary-adrenal axis, glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial and viral products. J. Endocrinol. *181*, 207–221.